Gothenburg, 27th May 2016

Dear Editor and Referees of SOIL Discussions

We thank you for the many constructive comments and corrections to our manuscript. We have used the input to improve the text. The details of our response and revision are given below.

First of all, we have clarified that the main aim of the paper is to present the new version of the ^{15}N tracing model *Ntrace*. The main advantage of this approach is the simultaneous quantification of rates in a more comprehensive model concept. For this reason we change the title to: 'Simultaneous quantification of depolymerization and mineralization rates by a novel ^{15}N tracing model', and the abstract is more focused written now:

'Abstract. Depolymerization of soil organic matter, such as proteins and (oligo-)peptides into monomers (e.g. amino acids) is currently considered to be the rate-limiting step for nitrogen (N) availability in terrestrial ecosystems. Mineralization of free amino acids (FAA), liberated by depolymerization of peptides, is an important fraction of total mineralization of organic N. Hence, accurate assessment of peptide depolymerization and FAA mineralization rates is important in order to gain a better process-based understanding of the soil N cycle. In this paper, we present an extended numerical ^{15}N tracing model *Ntrace*, which incorporates the FAA pool and related N processes in order to provide a more robust and simultaneous quantification of depolymerization and gross mineralization rates of FAAs and soil organic N. We discuss analytical and numerical approaches for two forest soils; suggest improvements of the experimental work for future studies; and conclude that: i) FAA mineralization can be an equally important rate limiting step for total gross N mineralization as peptide depolymerization rate, when about half of all depolymerized peptide N is directly mineralized; and that ii) gross FAA mineralization and FAA immobilization rates can be used to develop FAA use efficiency (NUE_{FAA}), which can reveal microbial N or C limitation.'

Some of the comments were general across more than one referee (R) and these are treated together (I, II, II and IV below) in a joint **reply to all referees**:

I. Including microbial biomass: all three reviewers have an opinion about this matter: **R3**: *'the current numerical model is missing a microbial biomass pool with a different turnover time than the SON pool'*; **R1**: *'the authors of this study do not include microbial biomass as an explicit pool in their model'*; **R1**: *'Methods page 7, lines 4-5: The underlying concept of a microbial N pool in Mooshammer et al. however also allows for the interpretation that any changes in these dynamics might be caused by changes in the microbial N pool. This interpretation is not possible with the Ntrace model'*, **R1**: *'It would be interesting to compare the numeric model with the analytical approach at lower amino acid amendments. This might also help to evaluate if it is necessary to include an explicit microbial N pool in models for soil N dynamics.'*, and **R1**: *'page 8, lines 21-26: When microbes are included in the interpretation of these results, it could also mean, that the addition of amino acids led to an increased uptake of amino acids but also an increased release of excess N as NH4 from the microbial biomass. Together with a potential down-regulation of peptidase activity this could be the reason for the observed results. Page 9, lines 1-6: In this model the MSON pathway is*

relevant, when changes in the NH4 pool cannot fully be described by the changes in the FAA pool. If a microbial pool was included in the model, changes in this pool, which should be situated between FAA and NH4 could be responsible for the observed dynamics. ' **R2**: *'It strikes me as odd that SON was not separated into microbial biomass and non-biomass pools. Any N "immobilized" into the non-biomass pool will be misrepresented as N assimilated by the microbial biomass and thus misrepresent NUE as the term is commonly understood. Along with this, measuring the 15N incorporated into the microbial biomass (e.g., using chloroform fumigation) would have been a helpful addition'*. **R3:** *'I agree with Referee #1 and #2 that the current numerical model is missing a microbial biomass pool with a different turnover time than the SON pool.'*

Our reply: True, we have not included microbial biomass explicitly in our conceptual model and not in the conducted experimental work. We see some challenges in incorporating a microbial N pool in the model and are not convinced that it will enhance the robustness of gross rate quantifications. The main challenge is that there exists no solid method to quantify active microbial biomass. The problem with the chloroform fumigation method (that R2 suggests) is that an extractability factor must be used in order to come to a value for the microbial biomass. This factor is in reality variable in different soils and soil depths and different extractants (Jörgensen and Müller, 1996). Historically, the factor for nitrogen (K_{EN}) is obtained by calibrating against the parallel factor for carbon (K_{EC}) (Jörgensen, 1996), which was originally calibrated with an incubation yielding a CO_2 measure from (inoculated) soil respiration. We believe, due to the uncertainty of K_{EN} and the chloroform fumigation method, that adding the microbial biomass to the model would complicate the set up unnecessarily and add uncertainty that later would be amplified in the model. Davidson et al. (1991) have stated that: *"As an alternative method, a non-linear equation is given for calculating the gross immobilization rate from the appearance of ^{15}N in chloroform-labile microbial biomass; but incomplete extraction of biomass N may result in low estimates"*.

Even if we could get a good measure of how many microbes are abundant in the soil, we would not know how many are active in assimilating N. Most of them are probably not active at all (Vandewerf and Verstraete, 1987). Therefore, to just measure the microbial biomass and incorporate it in the *Ntrace* model (like in model 1) would not improve the quantifications but in contrast lead to erroneous results.

In fact, although microbial N was measured in Mooshammer et al. (2014) they did not include microbial N in their model, neither in the calculations of gross rates or NUE.

In reply to R2: The immobilization is from our point of view, in our experimental setup, identical to the assimilation in microbial biomass, which due to turnover of microbes then becomes non-living SON (microbial residue N). The only other option in a closed soil incubation without plant roots, is adsorption to soil particles.

Again, R1: *'When microbes are included in the interpretation of these results, it could also mean, that the addition of amino acids led to an increased uptake of amino acids but also an increased release of excess N as NH4 from the microbial biomass….. At the very least the authors should discuss how an explicit microbial pool might change their model.'*

Our reply: To answer this comment we should look at the concept of nitrogen mineralization (Fig. I). Monomeric organic N (particularly FAA) is taken up by the microorganisms. A part of that N is used in the biosynthesis of microbial biomass, while the N exceeding the demand of biosynthesis is liberated as NH_4^+ inside the cell and then exuded to the soil solution. This NH_4^+ is though not mixed with the microbial N and consequently will still carry the same ^{15}N enrichment as the N taken up.

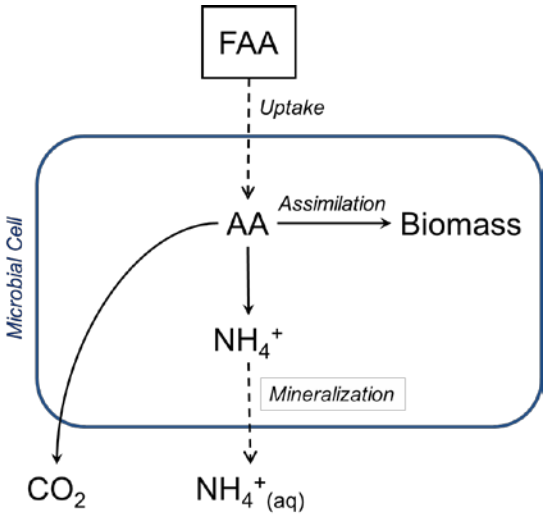

Figure I. Microbial mineralization of FAA (modified from Reddy & DeLaune, 2008).

Do we need to consider microbial N pool in a ^{15}N tracing model to realistically represent this dynamics? For evaluating that, we compare to models (Fig. II) for a situation assuming the following soil N contents (realistic proportions, arbitrary units): $[NH_4^+]$ = 20; [FAA] = 5 and $[N_{mic}]$ = 500. Labelling with ^{15}N enriched amino acids leads to a ^{15}N enrichment of FAA of 20 %. We assume that1 FAA is taken up, of which half is assimilated (immobilized) and half mineralized. In the following we illustrate with this example the reason we think model 1 is erroneous.

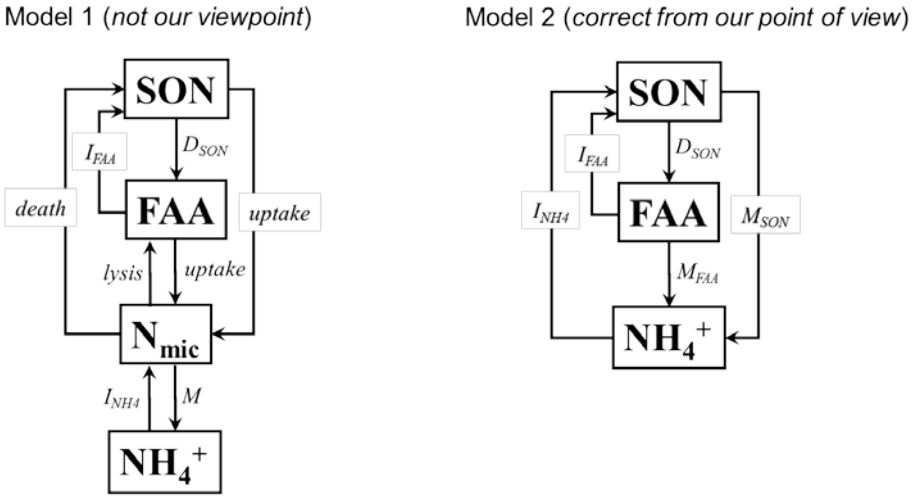

Figure II. Two models for mineralization.

Model 1 – Microbial: the FAA taken up by microbes is 20 % enriched in ^{15}N. If 1 FAA is taken up, the overall enrichment of the microbial N (prior to mineralization) is 0.04 % [= (1*0.2)/500]. In this model the mineralized N will originate from the microbial N pool and, hence, the NH_4^+ released by mineralization will have a ^{15}N enrichment of 0.04 %. This results in a ^{15}N enrichment of soil NH_4^+ of 0.001 % [= (0.5*0.0004)/20].

Model 2 – Implicit (as *Ntrace*): In that case, the mineralization of FAA directly transfers FAA-N to NH_4^+, and the ^{15}N enrichment of mineralized N will be the same as for the FAA, that is 20 %. This gives a ^{15}N enrichment of soil NH_4^+ of 0.5 % [= (0.5*0.2)/20].

Model 2 considers the fact that the mineralized FAA does not go through the microbial biomass and that the released NH_4^+ will still carry the ^{15}N enrichment of the FAA. This is consistent with the situation that is actually occurring, as illustrated in Fig. II. For this reason, model 2 is a more realistic scenario. As shown, considering a microbial N pool would lead to an erroneous quantification of gross mineralization. Theoretically, explicitly considering and measuring microbial N could improve the quantification of N immobilization, but as sated above this is hindered methodological issues on measuring the microbial N (see also Davidson et al., 1991).

II. Addition of ^{15}N-enriched amino acids. R1: '*The much more severe problem with the experimental setup is the enormous input of amino acids*', **R1**: '*By introducing this flush of amino acids, peptidases could be inhibited by their potential product and peptidase expression could be down-regulated due to the surplus of amino acids, which would result in lower depolymerization rates later in the incubation.*', and **R1**: '*It is also not clear to me why the authors chose these high amino acid amendments. The method described by Wanek et al. was developed for leaf litter, which can be expected to have much higher FAA concentrations. Also Wanek et al. mention twice in their paper that FAA concentrations should be determined beforehand and only 25% of the amino acid pool should be amended to avoid the effects the authors of this study discuss.*', and '*Results and discussion: Page 7 lines 16 to 17: as mentioned above, the integration over a longer time period does however not consider any physiological adaptations of the microbial community to the amino acid flush. Page 7, lines 17-19 and Page 9, lines 25-31: this problem has been addressed by Wanek et al., who suggested to determine the FAA pool and only amend 25% of that pool.'; and 'Page 8, lines 5-6: This might again be caused by the large amounts of amended amino acids'. And **R3**: 'My main concern relates to the high amount of 15N-labelled AA to both soils: approx. 600% and 120% of the initial FAA pool was added as 15N-AA to the Podzol and Umbrisol (based on data given in Table 1). It is known that addition of a given substrate often stimulates its consumption, and thus it is common practice to add only a small fraction of the initial target pool when performing isotope pool dilution assays, in order to minimize such bias on gross rate estimates. Furthermore, the authors erroneously state "Following the recommendation by Wanek et al. (2010), the FAA label addition was 10 to 20 times larger than the initial FAA content in the original substrate (litter or soil)" (Pg 9 L25-26). However, Wanek et al. (2010) clearly state that a maximum of 25% of the initial FAA pool should be added in the form of 15N-tracer, based on a preliminary determination of the size of the FAA pool. …….. As*

already pointed out by Referee #1, the enormous 15N-AA input could result in end-product inhibition of peptidases, leading to lower depolymerization rates during longer incubations. Here, I would like to add that longer soil incubations often result in ammonium accumulation over time, due to the absence of plant roots, which would constantly remove a part of the soil nutrients. For example, such an ammonium accumulation was observed for the Podzol, as the ammonium concentration increased 3-fold during the 240h incubation (Figure 2a).'.

Our reply: We agree that the high amount of added amino acids was far from ideal, a fact that we already mentioned in the paper (p.9, line 30). We regret that we have wrongly quoted the Wanek et al. 2010 paper. Indeed, the authors recommend a maximum 25% addition of amino acids, related to the background amino acid content. Prior to the experiment we had knowledge on the abundance of amino acids from previous soil samplings in the same site and based on this we chose the amount for amino acid addition. However, it turned out that the soil samples used for the experiment had lower amino acids content than expected, leading to the high additions.

We have re-written the section '3.4 Suggested improvements of the laboratory method' to correctly express the recommendation by Wanek et al. The following sentence is deleted: 'Following the recommendation by Wanek et al. (2010), the FAA label addition was 10 to 20 times larger than the initial FAA content in the original substrate (litter or soil).' And replaced by these sentences: 'The FAA label addition was 10 to 20 times larger than the initial FAA content in the soil. Wanek et al. (2010) recommend adding maximum 25 % of the background amino acid content, but we were not able to reach the recommended level. This specification requires pre-knowledge of the FAA content in the soils.' This is in the section '3.4 Suggested improvements of the laboratory method', and is followed by this text: 'The addition of FAAs might cause an unintended 'hot-spot' effect (Kuzyakov and Blagodatskaya 2015) which stimulates depolymerization by priming (Schimel, 1996; Di et al., 2000). On the other hand, upon addition of high amount of amino acids, peptidases could be repressed (Vranova *et al.* 2013; Glenn *et al.* 1973). Therefore, future studies should apply lower amounts of FAA.

Furthermore, we delete this text: 'The analytical derived *MFAA* (data not presented, Eq. 4) was in both soils higher than *M* (Eq.1 or 3), which is irrational. This might have been caused by the different time frames or the stimulation of *MFAA* but not of *M* by the AA addition. In any case, numerical ^{15}N tracing models overcome such inconsistencies, as all gross rates are quantified simultaneously.'.

III. Nutrient use efficiency was discussed and Reviewer 3 points out that: **R3**: *'**the nitrogen use efficiency model** proposed in the present study (NUEFAA) is conceptually different than that by Mooshammer et al. (2014) (NUE)'*. **R3**: *'Furthermore, the authors claim that using gross rates computed by their numerical model (IFAA and MFAA) yields more accurate estimates of microbial nitrogen use efficiency compared to the model by Mooshammer et al. (2014). The authors raise two points: (1) the analytical approach yields less accurate rate estimates (but see comment above), and (2) Mooshammer et al. (2014) use gross N mineralization (M) instead of amino acid mineralization (MFAA). However, the nitrogen use*

efficiency model proposed in the present study (NUEFAA) is conceptually different than that by Mooshammer et al. (2014) (NUE). The authors estimate here amino acid-N use efficiency (NUEFAA) based on amino acid immobilization and amino acid mineralization, whereas Mooshammer et al. based their model on gross amino acid consumption rate as proxy for microbial organic N uptake, since proteins are the main N-containing compounds in soil and plant litter. Therefore, both NUEFAA and NUE models are conceptually justified and there is no evidence that using MFAA instead of M yields better estimates of microbial NUE. Indeed, estimates of microbial NUE could be improved by including also organic N compounds other than amino acids, which, however, remains a great analytical challenge.'

Our reply: We concur with R3 that there are different thoughts behind the NUE in Mooshammer's paper, the input data differ between the Mooshammer NUE and our NUE_{FAA} and therefore these two methods cannot be directly compared.

- For this reason we have adjusted the manuscript to not focus on this comparison. E.g. in the Abstract, the purpose has been focused to deal with the *Ntrace* model development, and not the NUE calculations, hence we have deleted this sentence: **'**2) suggest an amino acid N use efficiency (***NUE$_{FAA}$***) for soil microbes, which is a more realistic estimation of soil microbial NUE compared to the NUE estimated by analytical methods.' And modified the following sentence: 'ii) gross FAA mineralization and FAA immobilization rates can be used to develop FAA use efficiency (NUE_{FAA}), which can reveal microbial N and C limitation.''.
- We have deleted the following sentences from the manuscript introduction: 'The microbial N use efficiency (NUE) representing the balance between immobilization and mineralization, is regulated by the soil organic matter (SOM) quality, e.g. soil C to N ratio (Mooshammer et al. 2014). A soil carbon (C) to N (C/N) ratio of 20 is suggested as a breakpoint where NUE reach a maximum (Mooshammer et al. 2014), as a result of microbial retention of N due to N limitation (at high NUE). Contrastingly, high N mineralization leading to low NUE, results from C limitation (Mooshammer et al., 2014).'; . The text in the introduction concerning NUE is the following: 'Carbon or N limitation of microbes in a soil govern the direction of the soil N flow towards mineralization (N in excess) or immobilization (C in excess) (Robertson and Groffman, 2015).'…'Given the obtained amino acid immobilisation and amino acid mineralization rates, the FAA use efficiency (NUE_{FAA}) can indicate whether a C or N limitation is occurring.'
- We have deleted this part of methods section: 'results from analytical solution, Mooshammer et al. (2014) calculated ***NUE*** as:

  $$\boldsymbol{NUE} = (C_{FAA} - M) / C_{FAA} \qquad (6)$$

  Equation 6 implies that gross N mineralization derived from the analytical calculations is solely derived from FAA mineralization.', in order to avoid making the direct comparison.
- Figure 1 has been slightly changed to not have any formulas for NUE shown, the only is now in the text.

- We have deleted figure 6 that directly compared data points from the two ways of calculation. And deleted the analytical solution from Table 2.
- We have modified the discussion by deleting the following section:

  'The observed differences in gross N transformation rates are connected to differences in soil organic matter quality and properties of the microbial biomass (Farrell et al., 2014). The C/N ratios for the two investigated soils were near the breakpoint (C/N ratio of 20) suggested by Mooshammer et al. (2014), at which a change from C limitation to N limitation of the microbial community occur (Fig. 6). By using the gross rates from *Ntrace*, the $\boldsymbol{NUE_{FAA}}$s were 0.57 for Umbrisol and 0.60 for Podzol, which is smaller than expected from the relationship presented by Mooshammer et al. (2014) (Fig. 6). However, the *Ntrace* derived $\boldsymbol{NUE_{FAA}s}$ agree with the results from the analytical approach obtained from the longest time step (30 mins to 360 mins), but not for the shorter time steps (Table 2; Fig. 6). For Umbrisol the $\boldsymbol{NUE_{FAA}}$ from the analytical approach (Eq. 6) at the shorter time steps (30 min to 60 min and to 120 min) were higher and fell within the confidence interval from Mooshammer et al. (2014; Fig. 6). We account this to the fact that Eq. (6) uses gross FAA consumption rates quantified by the analytical approach. As it is well understood, this approach provides an overestimation of consumption rates (C_{FAA}), due to substrate addition (Schimel, 1996; Di et al., 2000), hereby, the ***NUE*** (Eq. 6) will be biased towards high values. The Podzol showed significant input to gross mineralization from other organic N than FAAs therefore, the ***NUE*** of Podzol derived from the analytical equation (Eq. 6) (time step 30 min to 120 min) was low. Consequently, $\boldsymbol{NUE_{FAA}}$ is ideally assessed by considering FAA mineralization explicitly (Eq. 5). If the true $\boldsymbol{NUE_{FAA}}$ is lower as we suggest from the *Ntrace* approach, it is likely that a larger portion of FAAs taken up by microbes is subsequently mineralized, than would be suggested from the line in Fig. 6. This challenges the understanding of the shift of soil C limitation to N limitation, however the two investigated soils can neither be termed as N or C limited.'
- We have now added a sentence to the results and discussion about the computed NUE_{FAA}: 'The C to N ratio for the two soils near 20, which indicates that the soils are at a tipping point for either C or N limitation, according to the concept from Mooshammer *at al.* (2014; Figure 1). Our result of amino acid nutrient use efficiency (NUE_{FAA}) was 0.57 for Umbrisol and 0.60 for Podzol, which point towards a carbon limitation in those soils, as we hypothesized.'.
- Finally we have modified the conclusions point ii) as follows: 'FAA mineralization and FAA immobilization rates can be used for assessing FAA use efficiency (NUE_{FAA}) and soil N limitation'.

Finally **R3:** '*Pg 2 L14: From a biological rather than mathematical perspective, I would say that low NUE leads to high N mineralization, and not that high N mineralization leads to low NUE.'*

Our reply: We agree, but this sentence was deleted and the topic brought into another sentence, see corrections above.

IV. Comparing *Ntrace* with analytical calculation R2: *'Model comparisons. I don't know if there is a 'right' way, but comparing rates from a zero-order analytical model with a mixed kinetic numerical model seems fraught. The attempt to determine an integrated rate for a given time period seems reasonable, but exactly how this was done is not described in much detail. In reality the rates given by the two model types agree quite well.'*. **R1**: *'Abstract page 1, lines 16-17: while stated here and repeatedly throughout the manuscript, that the numerical approach is superior to the analytical method, later in the manuscript (page 8, lines 12-13) it is argued that the numerical model is valid because it produces results for Dson that are similar to the analytical approach. This is contradictory.'* **R1**: *Page 7, line 24: Since both of the presented approaches have their limitations and are biased by the large amount of amended amino acids, I think it is not possible to tell which method is more realistic.'* **R3**: *'how to evaluate which approach yields more realistic rate estimates, which certainly cannot be concluded by simply comparing rates estimated by the two different approaches. I believe that both analytical and numerical models have disadvantages and limitations…. The authors also claim that their numerical model overcomes the problem of high label addition by integrating gross rates over a longer time……. Therefore, I suggest that the authors need to provide some experimental evidence that the numerical model actually overcomes problems such as high label addition. Regarding the comparison between the two different approaches, statistical support should be provided for differences in rates estimated by the two models.'*.

Our reply: We concur there were some faults in the phrasing and logic for comparing the obtained rates. In the abstract we have now specified the outcome of the paper as follows: 'In this paper, we present an extended numerical ^{15}N tracing model *Ntrace*, which incorporates the FAA pool and related N processes in order to provide a more robust and simultaneous quantification of gross production and mineralization rates of FAAs together with gross N mineralization.'. Furthermore, we have removed this sentence from the abstract: 'Due to the short time span, soil disturbance and unnatural high FAA content during the first few hours after the labelling with the traditional ^{15}N pool dilution experiments, analytical models might overestimate peptide depolymerization rate.'

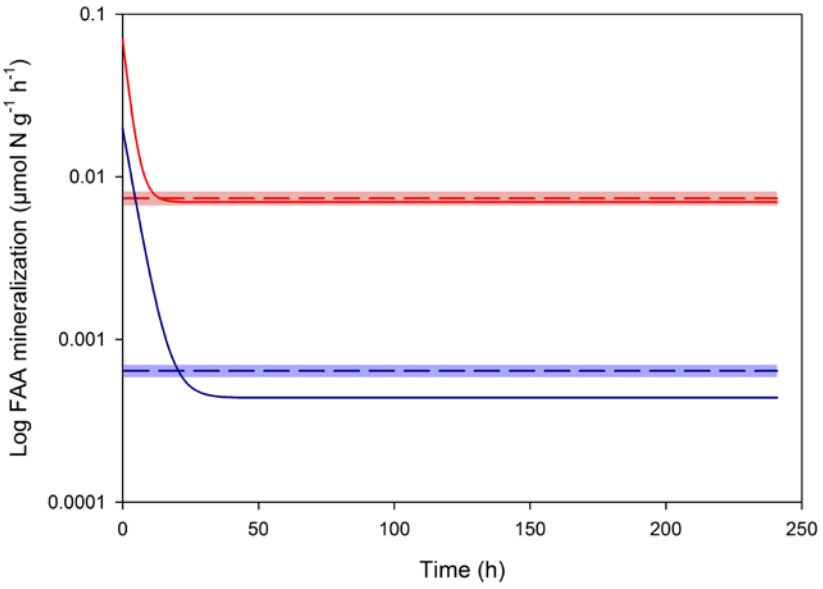

Figure III. Time course of FAA mineralization (M_{FAA}) over the 240 hours experimental duration (solid lines) for Umbrisol (red) and Podzol (blue), compared to the average rate (dashed lines; coloured areas ± standard deviation).

Figure III shows with an example from our data, how the *Ntrace* obtained M_{FAA} rate after a longer term incubation is a good estimate of the average rate. However, when calculated at the first hours of the experiment (as done in the analytical model approach) the rates are over estimated.

Regarding the issue of comparing *Ntrace* with analytical calculation we do not think it is contradictory to state that the numerical model is superior, and at the same time validate our model against analytical model results. Any robust numerical method should give the same results as an analytical model (if the same model structure is used), therefore having similar D_{SON} or total mineralization indicates that the numerical method is valid. Analytical models can be useful, and they are correct in terms of the mathematical integration, however numerical models are more robust as they provide estimation of all rates simultaneously and not sequentially. Therefore the output is not an exact solution of the equations but in fact an approximation to the exact mathematical solution. Another advantage is that numerical models have a coherent model concept, which means that we consider processes rather than total production and consumption. Myrold and Tiedje (1986) stated *"The structure of the N cycle makes it amenable to description as a compartmental system. The compartments are the pools of chemically or biologically distinct forms of N and the flows among these pools are the rates of the various N cycle processes."* Gross rates for consuming processes are also more realistic, as we go beyond the phase of rate stimulation (see Fig. II) and can use the model for experiments over longer time, not just 24 hours (which explains the particular differences in those compared to the similar production rates). In numerical models we can also use flexible kinetics as there is no reason why we would expect zero order kinetics for most processes. A more detailed model description of *Ntrace* and comparison with other analytical and numerical tracing models can be found in Rütting and Müller (2007).

R3: *'The excessive addition of 15N-AA in the present study likely resulted in biased gross rate estimates, in particular CFAA, and thus also affected the calculation of nitrogen use efficiency (NUE). The stimulation of amino acid mineralization rates due to high 15N-AA label addition, are also likely to have resulted in an overestimation of MFAA rates with the numerical approach (and not only rates estimated by the analytical approach), which would explain the high MFAA rates estimated.'*

Our reply: (see also the joint reply to the topic given above) The problem of stimulation of consumption processes will also occur in numerical tracing model, but only at the start of the experiment, as long as the substrate is elevated compared to background. As numerical tracing model integrate rates over longer time periods, this stimulation will be minimized. Indeed, as can be seen in Fig. 5, M_{FAA} was enhanced when estimated for the first 6 hours compared to the entire experimental duration of 240 hours. This points to the fact that the gross consumption rates can in general be quantified unaffected by substrate addition when integrated over longer time periods (but see also the discussion about our high substrate addition). Note also that the AA consumptions quantified by *Ntrace* are indeed lower compared to the analytical rates (Figure II)

Some additional referee comments were raised:

Anonymous referee R1:

1. '*The introduction should be concluded with concrete testable hypotheses, similar to those presented in the abstract. These hypotheses should be revisited in the discussion*.'

Our reply: Thank you for this suggestion, we have at the first introduction section now placed one research question: '…availability, hence our research question is whether the peptide depolymerization and FAA mineralization rates are two equally important steps co-limiting for N availability.', and at the end of introduction rephrased and added two hypothesis: 'In this paper we combine two parallel ^{15}N tracing experiments, in which soil is separately amended with ^{15}N labelled ammonium or an amino acid mixture. By splitting the amino acid labelled incubation, two rates (depolymerization rate and amino acid mineralization rate) were assessed from one label. For data analysis, we further developed the numerical ^{15}N tracing model *Ntrace* (Müller et al., 2007) to explicitly account for FAA turnover, in order to simultaneously quantify gross peptide depolymerization, gross FAA mineralization and total gross N mineralization in forest soils. For our selected mineral soils from Swedish spruce forest, our hypotheses are: 1) FAA mineralization is a major important part of gross N mineralization; 2) due to year-long successful forestry in this area we expect the soil to be carbon limited rather than N limited.'; In the results and discussion we revisit these hypothesis: '….that amino acid mineralization rate is a major part of the gross N mineralization as hypothesized, and can be considered as a co-limiting step for plant N availability in terrestrial ecosystems.', and: 'The C to N ratio for the two soils near 20, which indicates that the soils are at a tipping point for either C or N limitation, according to the concept from Mooshammer *at al.* (2014; Figure 1). Our result of amino acid nutrient use

efficiency (NUE_{FAA}) was 0.57 for Umbrisol and 0.60 for Podzol, which point towards a carbon limitation in those soils, as we hypothesized.'

2. '*Page 2, line 23-24: please state some of these obvious limitations ('These approaches apply analytical calculations (Kirkham and Bartholomew, 1954; Watkins and Barraclough, 1996) handling one flux at the time, which has some obvious limitations (Rütting et al. 2011).')'*

Our reply: We specify this as follows in the introduction: ' … limitations: 1. The analytical solutions only provide total consumption and production rates and not the specific processes, 2. analytical solutions only consider zero-order kinetics, 3. the possibility of re-mineralization / re-mobilization limits the experimental work to short time steps, finally 4. with the analytical approach gross rates are sequentially quantified, which does not take into consideration possible interactions; hence, the numerical modelling provides a more coherent framework as the process rates are quantified simultaneously (Rütting et al., 2011).'.

3. '*Page 10 lines 12-14: Especially for the amino acid pool dilution method a longer incubation time of 6H might result in problems with recycled labelled N.*'

Our reply: we change our recommendation slightly and delete the brackets: '(e.g. after 6 h)'.

4. '*Figures: Please stat for all figures that include error bars what these are and what the sample size was.*'

Our reply: This is now specified in the figure caption for: Figure 2: '..symbols indicate data observation with standard deviation (n = 5; except ^{15}N fraction of free amino acids: n = 4 at 13 min),… ', Figure 3: '…with standard deviation (n = 5; except ^{15}N fraction of free amino acids: n = 3 at 13 min)', Figure 4: 'Initial soil content of individual amino acids (µg N-FAA g^{-1} DW soil) indicated as average ± standard error (n = 5).', and Figure 5: '…mineralization rates [ng N g^{-1} h^{-1}] indicated as average with deviation (n = 5) for Umbrisol (A) and Podzol (B) ….. total) in [ng N g^{-1} h^{-1}] as average with standard deviation (n = 5),….'.

5. '*Figure 1. The second formula for NUE should have IFAA + MFAA in the denominator.*'

Our reply: This is correct, however we have deleted both formulas from the figure since we now only work with one formula in the paper.

<u>Anonymous referee R2</u>:

1. '*Table 1. It is peculiar that soil ammonium concentration is not provided. If available (and it should be), it should be added. It is important for the reader to know how the amount of 15N-label added compares to the natural background.*'

Our reply: We have provided the ammonium data. NH_4 concentration in µg g^{-1} DW soil (average and standard error) was for Podzol: 1.4 ± 0. 6 and for Umbrisol: 1.1 ± 0.9. These data are added to Table 1.

2. *'Units. Although maybe not the best, most 15N tracer studies provide concentrations and rates in terms of mass of N (e.g. mg, ug) rather than moles N. I would suggest tables and figures be converted to mass of N to make the data easily comparable to previous studies. Also rates are most often 'per day' rather than 'per hour'.'*

Our reply: We have changed the units to ngN g^{-1} h^{-1} in Figure 2, 3 and 5 and Table 2, and in the figure captions.

3. *'…types agree quite well (Table 2) except for CFAA, which leads me to question the validity of Eqn 4. I was too lazy too check Barracloughs derviation, but it makes me wonder if there is a flaw in Eqn. 4. Could the averaging to get the a' values be a factor?'*

Our reply: Originally the equation was developed by Watkins and Barraclough (1996) for plant residues, and in that case the added plant material had a constant ^{15}N excess (as no new residue was formed) that was used for the calculation. However, as in the case of amino acids the ^{15}N excess changes over time (due to production of AAs), we rather use the average ^{15}N excess of the two points. This averaging is similar to what Huygens et al. (2008) did for DNRA quantification (which is based on a similar thinking, see supplementary material).

Specific corrections

4. *'P1 l.8 use commas to set off the 'such as' phrase. I also wonder if monomers is too limiting ? It was the authors measure in this research, however, others (Farrell et al. I believe) have shown that small oligopeptides are preferred over amino acids.'*

Our reply: We have modified the first sentence as follows: 'Depolymerization of soil organic matter, such as proteins and (oligo-) peptides into monomers (e.g. amino acids) is currently considered to be the rate-limiting step for nitrogen (N) availability in terrestrial ecosystems.'

5. *'P2 l 15: A good classical reference that would fit here is Jansson and Persson 1982. Mineralization and immobilization of soil nitrogen p 229-252. In Steveson (ed.) Nitrogen in agricultural soils. ASA Madison, WI.'*

Our reply: We were not able to find this reference, but we have added this one instead: Robertson and Groffman 2015;. 'Carbon or N limitation of microbes in a soil governs the direction of the soil N flow towards mineralization (N in excess) or immobilization (C in excess) (Robertson and Groffman, 2015).' For the reference list: Robertson, G.P. and Groffman, P.M., Nitrogen transformations. Cpt. 14 in Soil microbiology, ecology, and biochemistry. Ed. Paul, E.A. 4th edition, Academic Press, Elsevier. 2015.

6. *'P2 l 15: delete 'to address', P 2 l 19 'thereby' rather than 'hereby'*

Our reply: Corrected accordingly.

7. *'P 2 l 31: define NUEFAA.'*

Our reply: The sentence was deleted.

8. *‘P3 l 9: parantheses around the year. P3 l 15 the #th# by dazes can be deleted. P3 l 24: capitalize Laboratories.’*

Our reply: Corrected accordingly.

9. ‘*P4 l 4: provide the rationale for the calcium sulfate / formaldehyde extraction. It is not a standard method that readers will know.’*

Our reply: We have added this sentence to the text: ‘The $CaSO_4$ was selected because deionized water alone lyses microbial cells, thereby releasing a large flux of amino acids from the cells. Formaldehyde was used in order to inhibit microbial consumption or activity during the shaking time.’

10. ‘*P 4 l 25: it is more typical to oven dry mineral soils at 105 C; 75 C is more normal for plant tissue (or organic soil horizons).’*

Our reply: We agree that this is classical, but as we wanted to analyse the same soil samples for total C and N, we did not want to risk losing volatile organic matter (C and N) during the drying procedure, for this reason we prefer 75 deg. C.

11. *‘P 5 l 5 The Andresen ref is inappropriate here as the equations come directly from the original Kirkham and Bartholomew paper*.’

Our reply: we have removed the reference.

12. ‘*P5 l 18 Are you sure it is logarithmic, or was it exponential ?’*

Our reply: the functions are indeed logarithmic (example: M, $mg\ N\ kg^{-1}\ hr^{-1}$ = -0.023ln(x, *hr*) + 0.169).

13. ‘*P 6 l 6: delete the comma after ‘could’.’*

Our reply: we have removed it.

14. ‘*P6 l 8 I don’t think the pool is ‘infinite’, it is large and changes imperceptibly during the short incubation period’*

Our reply: We have rephrased this: ‘The N transformations were either implemented as zero-order kinetics for large substrate pools that is constant in size during the incubation (D_{SON} and M_{SON}) or first-order kinetics for finite pools (M_{FAA}, I_{FAA} and I_{NH4}).’

15. ‘*P 6 l 28 add % after ‘22’.’*

Our reply: Corrected accordingly. .

16. ‘*P 7 l 15 this seems like a throw away sentence as it does not really lead to greater understanding of the results.’*

Our reply: We have rephrased the sentences: ‘Numerical tracing models represent robust methods to assess gross transformation rates, as all data points from the two isotope label experiments and all observed time steps are included. To our knowledge, quantification of

total gross FAA mineralization and peptide depolymerization rates had not been done by numerical tracing models.'.

17. *'P 7 l 18 problem of additions stimulating processes is as true regardless of what approach one uses to analyse the data. Now, it is true that if the model uses first-order kinetics, then this "mass dependency" is accounted for to some degree, but one can incorporate these kinetics in the analytical model (see "Case 2" in the rarely quoted 1955 paper by Kirkham and Bartholomew).*'

Our reply: Indeed, Kirkham and Bartholomew developed in their 1955 paper an analytical model using first-order kinetics for NH_4 consumption. However, this model assumes that all NH_4 is immobilized and none is nitrified, i.e. that a closed N cycling between SON and NH_4 exists. This will almost never be the case, for which reason that first-order analytical model is almost never applicable.

18. '*P 7 l 16 correct to 'assess'.*'

Our reply: This is done.

19. '*P 8 l 6 Is "irrational" the best word? The result is illogical, but that raises a question as to whether there is a flaw in the logic behind equation 4?*'

Our reply: The equation 4 is indeed correct, see reply to R1, but the mentioned sentence was deleted during responding to R1.

20. '*P 10 l 5 change to 'points'.*'

Our reply: This is done.

21. '*P 10 l 26 Chitin is not an amino acid, it is an amino sugar polymer.*'

Our reply: this is now specified: 'Another outlook is that depolymerization rates of polymers other than amino acids (such as amino sugar polymers) are potentially an important part of the total depolymerization.'.

22. '*Capitalization of titles is inconsistent (particularly older references)*'

Our reply: This is corrected accordingly.

<u>Anonymous referee R3</u>:

1. *'In Figure 2d and 3d, it becomes also evident that the 15N-AA label was not homogenously distributed, otherwise there would have not been an increase in 15N/14N in the first time interval. Of course, for this time interval, it is not possible to calculate a rate using the analytical approach. The weak dilution of the 15N-AA label during the first time intervals was not due to low depolymerization rates, as suggest by the authors, but rather likely due to high 15N-labelled substrate addition, which resulted in an enrichment of the FAA pool of 60-70 at%.'*

Our reply: We assign this slight (insignificant) increase in ^{15}N rather to heterogeneity of the soils, even though that homogenized samples were used. The high ^{15}N enrichment should rather increase the sensitivity to detect small production, as low inflow of unlabelled material should lead to a more visible dilution of highly enriched pools compared to low enriched pool. We do not see how the high ^{15}N enrichment should lead to weak dilution. Therefore, we still conclude that the low dilution is indeed due to low depolymerization rate.

2.*'Equations of the analytical approach (Eq. 1, 2 and 3) are partially wrong: equation 1 for production rate has a mistake in the numerator of the second term; equation 2 for the consumption rate is actually the production rate; and, equation 3, for the case when there is no change in concentration over time, also seems wrong, at least when compared to their former work (Andresen et al. 2015).'*

Our reply: All three equations are correct. However, equations are slightly different presented compared to Andersen et al. (2015). While in the former paper we presented equations with the ^{15}N excess fraction (a) as input, here we use the equations analogous to the original Kirkham and Bartholomew paper using the ^{15}N excess amount (H) as input.

3. *'Furthermore, the authors used different units: µg N/g, µg FAA/g, µmol N/g, nmol N/g. In some instances, it is not even stated whether it refers to N or FAA (Table 1, Table 2). I suggest using consistent units throughout the manuscript. As I understood it, the initial FAA concentrations presented in Table 1 should correspond to the sum of FAA presented in Figure 4. For Podzol, Table 1 shows 1.3 µg, whereas in Figure 4 the values roughly sum up to 3 µg. In turn, for Umbrisol, both Table 1 and Figure 4 seems consistent: 7.7 µg in Table 1, as well as roughly 7.7 in Figure 4.'*

Our reply: We have now standardized this as much as possible: in Table 1 and Figure 4 the FAA unit is now both consistently µg N/g from FAAs, and specified this in the Table heading: 'and total free amino acid content (FAA in µg N g^{-1}DW).' And Figure caption: 'Figure 4. Initial soil content of individual amino acids (µg N-FAA g^{-1} DW soil) indicated as average ± standard error (n = 5). ', and axis title: 'Free amino acid content (µg N g^{-1})'.

4. '*Pg 1 L14: Comma is missing before which; Pg 1 L16: Delete "2)".'*

Our reply: Corrected accordingly.

5. *'Pg 2 L13: correct to "reaches".'*

Our reply: this whole sentence complex was removed, see other reply.

6. *'Pg 2 L23-24: The authors should state what the obvious limitations are.'*

Our reply: See reply above to R1.

7. *'Pg 4 L1-2: For N mineralization, 13 min for equilibration between added and native ammonium is quite short.'*

Our reply: The important information from the initial extraction is to what extend the NH_4 pool was labelled. The immobilization starts as soon as the NH_4^+ is added, therefore we aimed to keep this time as short as possible. Waiting too long between addition and first extraction leads to an underestimation of NH_4^+. Even though that a homogenous label distribution might

not have been achieved at that point (if it ever can), we do not see that this could be a major issue for the quantifications.

8. *'Pg 4 L3-4: Why were FAAs also extracted with 1 M KCL?'*

Our reply: The sample labelled with AAs was split in two: one sample was extracted with $CaSO_4$ solution and one was extracted with KCl. This was in order to measure $^{15}N\text{-}NH_4$ in the KCl sample. For clarification, the following text is added to the end of this section: 'The KCl extract was made for $^{15}N\text{-}NH_4$ analyses.'.

9. *'Pg 4: The authors used the protocol developed by Wanek et al. (2010). However, the authors do not even once cite this work in Materials & Methods.'*

Our reply: The reference to Wanek et al. 2010 is now appropriately added to the methods section.

10. *'Pg 4 L16: The reference is wrong. It should be Husek, 1991.'*

Our reply: The mistake is corrected.

11. *'Pg 4 L18-23: The authors should explain why some amino acids have the same m/z: Alanine and Glycine (m/z 116/117); Leucine, Serine, Isoleucine and Threonine (m/z 158/159); Proline and Aspartic acid (m/z 142/143)'*

Our reply: We thank the reviewer for nothing this error, the m/z given in the original manuscript were wrong, and is corrected now: 'Alanine (Ala m/z: 116/117), Glycine (Gly m/z: 102/103), Valine (Val m/z: 144/145), Leucine and Isoleucine (Leu and Ile m/z: 158/159), Serine (Ser m/z: 131/132), Threonine (Thr m/z: 146/147), Proline (Pro m/z: 142/143), Aspartic acid (Asp m/z: 188/189), Asparagine (Asn m/z: 141/143), Methionine (Met m/z: 249/250), Glutamic acid (Glu m/z: 202/203), Phenylalanine (Phe m/z: 192/193), Lysine (Lys m/z: 156/157), Tyrosine (Tyr m/z: 280/281) and Tryptophan (Trp m/z: 130/131).' (This replaces the following text in the methods section: 'Alanine and Glycine (Ala and Gly; m/z: 116/117), Valine (Val; m/z: 144/145), Leucine, Serine, Isoleucine and Threonine (Leu, Ser, Ile and Thr; m/z: 158/159), Proline and Aspartic acid (Pro and Asp; m/z: 142/143), Asparagine (Asn, m/z: 188/189), Methionine (Met, m/z: 249/250), Glutamic acid (Glu; m/z: 202/203), Phenylalanine (Phe; m/z: 192/193), Lysine (Lys; m/z:156/157), Tyrosine (Tyr; m/z: 280/281) and Tryptophan (Trp; m/z: 130/131)'). Only for Leucine and isoleucine (isomers) the same m/z was used (they are well separated chromatographically). The selected ion fragments are similar to those selected in Wanek et al. 2010, and were selected as N containing ion fragment with high intensity. In most cases the selected ion correspond to fragment resulting from the loss of $^{\bullet}CO_2CH_2CH_3$, though for some (methionine, lysine, threonine, histidine, tryptophan) another fragment had to be selected (due to low intensity or interfering fragments). We believe that giving the structure of the used ion fragments is beyond the scope of this paper.

12. *'Pg 5 L5 and L15: The original references (Kirkham and Bartholomew, 1954; Watkins and Barraclough, 1996) are sufficient. Delete reference Andresen et al., 2015.'*

Our reply: Done in both places.

13. *'Pg 5 L13: Specify that it is excess 15N abundance.'*

Our reply: We have corrected to: 'Excess ^{15}N content' (but this is not the same as abundance).

14. *'Pg 6 L15: I do not always see a good fit of the model in Figure 2 and 3. For example in Figure 3b, the fit of the model for ammonium concentration seems not to fit the experimental data.'*

Our reply: Indeed, the model does not lie within the uncertainty of all individual data points. However, importantly the overall performance of the model is quite well given that (1) the overall trends are well represented and (2) that the majority of data points are fitted by the model (particularly for data in Fig. 2). For the data in Fig. 3 (Podzol) the main challenge was the very low dilution of ^{15}N in the FAA, which will also affect how good the other pools are represented by the model. To achieve a better model fit in future studies, we suggest in the paper to have a later measurement point in the ^{15}N-AA labelling treatment to better follow the NH_4 pool and its ^{15}N enrichment (section 3.4 Suggested improvements of the laboratory method).

15. *'Pg 8 L18-19: In Wanek et al. (2010) the samples were plant litter and not organic soil.'*

Our reply: We have corrected the mistake and rephrased: 'The ratio of total gross N mineralization (*M*) to peptide depolymerization (D_{SON}) rate ranges from 5 to 25 % in both organic soils and plant litter, based on analytical calculations (Wanek et al., 2010; Wild et al., 2015).'

16. *'Figure 1 B: Equation is wrong.'*

Our reply: Equations were removed, see reply earlier to the common question of NUE.

17. *'Table 1: The initial soil ammonium concentrations of both soils should be stated. There is no need to say that the C:N ratio refers to dry soil.'*

Our reply: The ammonium data is added, se reply to R2. And reference to 'dry' is removed from the table heading.

18. *'Figure 4: Typo on the y-axis.'*

Our reply: Corrected.

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Simultaneous quantification of d~~D~~epolymerization and mineralization rates ~~– investigating N availability~~ by a novel ^{15}N tracing model

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Abstract. Depolymerization of soil organic matter, such as proteins and (oligo-)peptides into monomers (e.g. amino acids) is currently considered to be the rate-limiting step for nitrogen (N) availability in terrestrial ecosystems. Mineralization of free amino acids (FAA), liberated by depolymerization of peptides, is an important fraction of total mineralization of organic N. Hence, accurate assessment of peptide depolymerization and FAA mineralization rates is important in order to gain a better process-based understanding of the soil N cycle. In this paper, we present an extended numerical ^{15}N tracing model Ntrace, which incorporates the FAA pool and related N processes in order to provide a more robust and simultaneous quantification of depolymerization and gross mineralization rates of FAAs and soil organic N. We discuss analytical and numerical approaches for two forest soils; suggest improvements of the experimental work for future studies; and conclude that: i) FAA mineralization can be an equally important rate limiting step for total gross N mineralization as peptide depolymerization rate, when about half of all depolymerized peptide N is directly mineralized; and that ii) gross FAA mineralization and FAA immobilization rates can be used to develop FAA use efficiency (NUE_{FAA}), which can reveal microbial N or C limitation.

Keywords: Free amino acids, nitrogen, ^{15}N, numerical model, ~~microbial nutrient~~FAA use efficiency, amino acid mineralization, depolymerization rate

1. Introduction

Soil organic nitrogen (SON) mineralization is essentially a sequence of depolymerization of polymeric organic compounds followed by mineralization of the liberated monomers (Schimel and Bennett, 2004). Inorganic nitrogen (IN), such as nitrate

(NO_3^-) and ammonium (NH_4^+), as well as free amino acids (FAAs) are known to be the main plant N sources (Schimel and Chapin, 1996; Bardgett et al., 2003). Therefore, it is essential to know more about the production of mineral and amino acid N, and the balance between mineralization and immobilization of N, in order to have a better understanding of N availability. Gross N mineralization includes mineralization of FAAs, mineralization of other organic monomers and potentially also includes a share of NH_4^+ released from mineral complexes (Houlton and Morford 2015). It has been estimated across grassland, cropland and heathland ecosystems that FAA mineralization can be a substantial fraction of the N mineralization (34 % to 88 %; reviewed in Andresen et al., 2015). Hence, amino acid mineralization is important for IN availability hence, our research question is whether~~if~~ the peptide depolymerization and FAA mineralization rates are two equally important steps co-limiting for N availability.~~.~~

~~The microbial N use efficiency (NUE) representing the balance between immobilization and mineralization, is regulated by the soil organic matter (SOM) quality, e.g. soil C to N ratio (Mooshammer et al. 2014). A soil carbon (C) to N (C/N) ratio of 20 is suggested as a breakpoint where NUE reach a maximum (Mooshammer et al. 2014), as a result of microbial retention of N due to N limitation (at high NUE). Contrastingly, high N mineralization leading to low NUE, results from C limitation (Mooshammer et al., 2014).~~ Carbon or N limitation of microbes in a soil ~~is hence thought to~~ governs the direction of the soil N flow towards mineralization (N in excess) or immobilization (C in excess) (Robertson and Groffman, 2015). ~~However,~~As soil C/N ratio is a rather blunt measure for the C/N ratio of substrates used by microbes (potentially camouflaging inert mineral components, or recalcitrant soil organic matter),~~.~~ Schimel and Bennett (2004) suggested ~~to address~~ depolymerization rates, driven by extracellular enzymatic activity, as the rate limiting step for the terrestrial N cycle, thereby determining availability of the amino acid substrate within the soil and assessing the N release from the biologically available fraction in the soil. Following this, Wanek et al. (2010) provided methodological development of ^{15}N pool dilution essays to determine gross peptide depolymerization rates, and by combining this with ^{15}N tracing, quantification of gross FAA mineralization can in addition be achieved (Andresen et al., 2015). These approaches apply analytical calculations (Kirkham and Bartholomew, 1954; Watkins and Barraclough, 1996) handling one flux at the time, which has some ~~obvious~~ limitations: 1. The analytical solutions only provide total consumption and production rates and not the specific processes, 2. analytical solutions only consider zero-order kinetics, 3. the possibility of re-mineralization / re-mobilization limits the experimental work to short time steps, finally 4. with the analytical approach gross rates are sequentially quantified, which does not take into consideration possible interactions; hence, the numerical modelling provides a more coherent framework as the process rates are quantified simultaneously (Rütting et al., 2011)'~~limitations (Rütting et al. 2011)~~. To advance our understanding of the organic N dynamics and mineralization, we deemed it timely to present a novel numerical ^{15}N tracing model. Given the obtained amino acid immobilisation and amino acid mineralization rates, the FAA use efficiency (***NUE***$_{FAA}$) can indicate whether a C or N limitation is occurring~~relevant, as a more specific tool for soil quality assessment, than soil C to N ratio~~.

In this paper we combine~~, for the first time,~~ two parallel ^{15}N tracing experiments, in which soil is separately amended with ^{15}N labelled ammonium or an amino acid mixture. By splitting the amino acid labelled incubation, two rates (depolymerization rate and amino acid mineralization rate) were assessed from one label. For data analysis, we further developed the numerical ^{15}N tracing model *Ntrace* (Müller et al., 2007) to explicitly account for FAA turnover, in order to simultaneously quantify gross peptide depolymerization, gross FAA mineralization and total gross N mineralization in forest soils. ~~With this approach a more robust and coherent rate assessment and a more accurate calculation of microbial amino acid nutrient use efficiency (NUE_{FAA}) is achieved.~~ For our selected mineral soils from Swedish spruce forest, our hypotheses are: 1) ~~Furthermore, we discuss the importance of~~ FAA mineralization is a major important part of ~~for~~ gross N mineralization; 2) due to year-long forestry in this area we expect the soil to be carbon limited rather than N limited.~~ and present the peptide depolymerization and FAA mineralization rates as two important steps co-limiting for N availability in forest soils.~~

2. Methods

2.1 Field site

Soil was sampled from two forests at the Skogaryd Research Catchment part of SITES (Swedish Infrastructure for Ecosystem Studies, www.fieldsites.se), situated in southwest Sweden (58° 23'N, 12° 09'E; 60 m above sea level). Mean annual temperature is 6.4 °C and the mean annual precipitation is 709 mm (Ernfors et al. 2011). The soil of the first forest was an Umbrisol with sandy loam texture and was planted with Norway spruce (*Picea abies*) in the 1950s. The vegetation was classified as a spruce forest of low herb type based on the classification system by Påhlsson, (1998), with sparse ground vegetation dominated by bryophytes (*Mnium hornum*, *Polytricum formosum* and *Pleurozium schreberi*). The second forest was on a Podzol soil, where the vegetation was classified as a spruce forest of bilberry type (Påhlsson, 1998). The tree stand (Norway spruce) was 55-130 years old and of 23-30 m height. The ground vegetation was dominated by *Vaccinium myrtillus* and mosses.

2.2 Soil sampling

Soil was sampled with an auger on 14~~th~~ April 2014 (Umbrisol) and 12~~th~~ May 2014 (Podzol), each with five field replicates. The air temperature at both sampling times was 11º C. For the Umbrisol, the thin litter layer and vegetation was pushed aside and the soil was sampled until 10 cm depth. For the Podzol, the soil was sampled below the O-horizon and 10 cm down. These depths were selected to get matching low SOM contents. The soil was immediately transported to the lab, where roots and stones were manually removed. Wet soil (40 g) was placed in 250 mL glass bottles with a lid with a small hole, and pre-incubated for a week at constant temperature (20 °C) prior to labelling with ^{15}N.

2.3 ^{15}N labelling incubations

The pre-incubated soil was labelled with ^{15}N in two different treatments, either receiving $(^{15}NH_4)_2SO_4$ (99 % ^{15}N) or ^{15}N-amino acid mixture ('Cell Free' amino acid mix, 20 AA, U-^{15}N 96-98 %, chemical purity >98 %, Cambridge Isotope L~~l~~aboratories, USA). The total N addition with NH_4^+ was 0.6 µg N g^{-1} dry soil. The total added amino acids (AA) was 9.32 µg N g^{-1} dry soil (Umbrisol) or 7.72 µg N g^{-1} dry soil (Podzol). The label solution was added into the pre-incubated soil using a pipette (4 ml per bottle) and quickly stirred with a clean spatula.

Soils from NH_4^+ labelling was extracted using a 1 to 2 soil to liquid ratio, with 1 M KCl, by shaking for one hour at 120 rpm, then the samples were filtered (Whatman qualitative filter papers, No 1) and kept frozen (-18° C) until further processing. Soil extractions were done on one set of samples directly after labelling (13 min), the rest of the bottles were incubated in a dark room at constant temperature (20º C) until extraction after 24, 48, 96, 168 and 240 hours (h).

Soils from AA labelling were divided in two parts immediately after label addition, prior to incubation in the dark room. Simultaneously one half was extracted with 1 M KCl as described above, and the other half was extracted with 10 mM $CaSO_4$ containing 3.4% formaldehyde, in 1:2 soil to liquid ratio, by shaking for 1 h at 120 rpm, then the samples were filtered (Whatman qualitative filter papers, No. 1) and kept frozen until further processing. Extractions were done 13 min and 0.5, 1, 2 and 6 h after labelling. The $CaSO_4$ was selected because deionized water alone lyses microbial cells, thereby releasing a large flux of amino acids from the cells. Formaldehyde was used in order to inhibit microbial consumption or activity during the shaking time. The KCl extract was made for ^{15}N-NH_4 analyses.

2.4 Analysis of ^{15}N

All KCl extracts were prepared for analysis of ^{15}N contents of NH_4^+ by chemical conversion to N_2O and ~~analyzed~~analysed by IRMS (ANCA-TGII interfaced with a 20-20 IRMS, SerCon, UK) as described by Stevens and Laughlin (1994). NH_4^+ concentration was measured using a flow injection analyzer (Auto Analyzer 3, Bran+Luebbe, Norderstedt, Germany).

The $CaSO_4$ extracts for ^{15}N–AA analysis were purified using cation-exchange cartridges (OnGuard II H, 1 cc, Dionex), conditioned with ultrapure water (>18.2 MΩ), 3 M NH_3 and 1 M HCl. After loading the extract on the cation-exchange resin, the cartridge was washed with 10 mL ultrapure water and AAs were eluted with 30 mL 3 M NH_3. The purified sample was dried under reduced pressure at 35 °C, and finally derivatized using ethanol-pyridine and ethylchloroformate (Wanek et al., 2010; Husek et al., ~~2001~~1991). Finally the individual FAAs were measured by gas chromatography – mass spectrometry (GC – MS, Trace GC – DSQ, Thermo Fisher). Separation was done on a OV1701 column (30 m × 0.25 mm ID × 0.25 µm film; Sigma-Aldrich, Diegem, Belgium) for the 16 amino acids: Alanine ~~and Glycine~~ (Ala~~ and Gly;~~ m/z: 116/117), Glycine (Gly m/z: 102/103), Valine (Val~~;~~ m/z: 144/145), Leucine and~~, Serine,~~ Isoleucine ~~and Threonine~~ (Leu and Ile~~, Ser, Ile and Thr;~~ m/z: 158/159), Serine (Ser m/z: 131/132), Threonine (Thr m/z: 146/147), Proline ~~and Aspartic acid~~ (Pro~~ and Asp;~~ m/z: 142/143), Aspartic acid (Asp m/z: 188/189), Asparagine (Asn~~,~~ m/z: ~~188/189~~141/143), Methionine (Met~~,~~ m/z: 249/250), Glutamic acid (Glu~~;~~ m/z: 202/203), Phenylalanine (Phe~~;~~ m/z: 192/193),

Lysine (Lys; m/z: 156/157), Tyrosine (Tyr; m/z: 280/281) and Tryptophan (Trp; m/z: 130/131). The following amino acids were not possible to measure: Arginine, Glutamine, Histidine and Cysteine.

2.5 Soil properties

Soil water content was determined gravimetrically (GWC) by oven drying of c. 10 g soil samples to constant weight at 75 °C. Soil pH was measured in 1 M KCl extracts. Soil organic matter was determined on 2 g of dried soil samples by loss of ignition (8 h at 500 °C). Total soil N and C content was determined on ground soil with an elemental analyzer (ANCA SerCon, Crew, UK).

2.6 Calculations

2.6.1 Analytical equations

In order to quantify the gross N mineralization (M), NH_4^+ consumption (C_{NH4}), FAA consumption (C_{FAA}) and peptide depolymerization rates (D_{SON}; Fig. 1) the analytical equations developed for isotope pool dilution experiments were used (Kirkham and Bartholomew, 1954; Andresen et al., 2015):

For $p > c$:

$$p = \frac{M_t - M_0}{t} \times \frac{\ln(H_0 M_t / H_t M_0)}{\ln(M_t / M_0)} \quad (1)$$

$$c = \frac{M_t - M_0}{t} \times \frac{\ln(H_0 / H_t)}{\ln(M_t / M_0)} \quad (2)$$

For $p = c$

$$c = p = \frac{M_{av}}{t} \times \ln\left(\frac{H_0}{H_t}\right) \quad (3)$$

Where p = production rate (i.e. M or D_{SON}; respectively), c = consumption rate (i.e. C_{NH4} or C_{FAA}, respectively), M_i = total content of labelled pool, H_i = excess ^{15}N content of labelled pool. Indices i indicate: initial (0), final (t) and average (av) content.

In order to quantify the FAA mineralization rate (M_{FAA}; Fig. 1), the following equations were used (Watkins and Barraclough, 1996; Andresen et al., 2015):

$$M_{FAA} = p * \frac{a'_1 * (M_t / M_0)^{\frac{p}{\theta}} - a'_0}{a'_{aa} * (M_t / M_0)^{\frac{p}{\theta}} - a'_{aa}} \quad (4)$$

With p being gross mineralization rate obtained from Eq. (1) or Eq. (3) from the ^{15}N-NH_4^+ labelling experiment, extrapolated to 0-6 h by a logarithmic function; θ is $(M_t - M_0)/t$ with M = NH_4^+ content from the ^{15}N-AA labelling; a'_{aa} is the excess ^{15}N

fraction of the total FAAs pool averaged for the two time steps; a' is the excess ^{15}N fractions of the NH_4^+ pool from the ^{15}N-AA labelling.

2.6.2 Iterative numerical model *Ntrace*

Numerical ^{15}N tracing models have been used to investigate soil inorganic N dynamics (Myrold and Tiedje, 1986; Rütting et al., 2011). Among the main advantages of a numerical approach is that process specific gross N transformation rates are quantified simultaneously rather than sequentially (Rütting and Müller, 2007). Therefore, interactions between N transformations are accounted for. Here we further developed the ^{15}N tracing model *Ntrace* (Müller et al., 2007) to explicitly include FAA dynamics (Fig. 1). The mineralization of complex soil organic matter is represented as a two-step process: 1) peptide depolymerization releasing free AAs (FAA) (depolymerization rate D_{SON}), and 2) mineralization of FAA to NH_4^+ (amino acid mineralization rate M_{FAA}). In addition, mineralization of other (non-peptide OR non-AA-polymers) SON (M_{SON}) to NH_4^+ was included, which accounts for depolymerization followed by mineralization of other N compounds (e.g. chitin; Bai *et al.*, 2013). Gross N mineralization is hence the sum of M_{FAA} and M_{SON}. Immobilization of FAAs (I_{FAA}) and ammonium (I_{NH4}) is also included in the model. In the current study, $^{15}NO_3^-$ could not be measured even after addition of $^{15}NO_3^-$, due to too low NO_3^- content of the soil. Therefore, oxidation and immobilization of NH_4^+ could~~,~~ not be separated and the quantified gross NH_4^+ immobilization (I_{NH4}) is the sum of these two processes. The N transformations were either implemented as zero-order kinetics for large substrate pools that is constant in size during the incubation (D_{SON} and M_{SON}) or first-order kinetics for finite pools (M_{FAA}, I_{FAA} and I_{NH4}).~~The N transformations were either implemented as zero-order kinetics for infinite substrate pools (D_{SON} and M_{SON}) or first-order kinetics for finite pools (M_{FAA}, I_{FAA} and I_{NH4})~~.

A Markov chain Monte Carlo sampling was used for parameter estimation by fitting the model to measured contents and ^{15}N enrichments of the studied pools (Müller et al., 2007). The outcome is a probability density function for each model parameter, from which parameter averages and standard deviations can be calculated (Rütting and Müller, 2007). For D_{SON} in the Podzol, the probability density function was truncated at zero. Therefore, average and standard deviation for that parameter were calculated using functions for truncated normal distributions (Cohen and Woodward, 1953; Cicchinelli, 1965). For N transformations described by first-order kinetics, average gross rates were calculated by integrating the gross rates over the experimental period. A good fit of the model to the experimental data was achieved (Figs. 2 and 3).

A mix of 20 different amino acids was added to the soil. However, four of the added AAs (Arginine, Cysteine, Glutamine and Histidine) could not be measured with the current methodology. The N of these four AAs accounted for 22 % of the added ^{15}N in the experiment. As those AAs also contribute to the mineralization (^{15}N-NH_4^+ production), these were considered in the tracing model as follows: we assume that the soil pool of non-measured AAs has the same average ^{15}N enrichment as the pool of the measurable 16 AAs. The pool of non-measured AAs was then included, having the same depolymerization, mineralization and immobilization rates as the measured AAs. In order to evaluate the potential effect of the assumption of the same ^{15}N enrichment, an uncertainty data analysis with altered ^{15}N enrichment for the missing AAs

was conducted, which indicated that altered ^{15}N enrichment had only marginal effects on the estimated gross rates (see Supplement Table S1). We argue that the most realistic gross rates are quantified when including the non-measured AAs. However, in order to compare the results from the *Ntrace* with the analytical rates, M_{FAA} and I_{FAA} were additionally calculated for the measured AAs only, either for the entire incubation period ('240 h') or for the first 6 h only (same time-frame as for analytical calculations). The gross rates including all AAs will be higher in proportion to the amount of AA-N (i.e. 22 %, when compared to rates for measurable AAs only).

2.6.3 Nitrogen use efficiency

Microbial N use efficiency of free amino acids (***NUE***~~; Fig. 1~~) is the fraction of consumed FAAs that is not released as ammonium but incorporated into the microbial biomass (Mooshammer et al., 2014). We calculated ***NUE*** specifically for amino acids (***NUE***$_{FAA}$) by another formula that used by Mooshammer et al., (2014) based on the *Ntrace* results as:

$$NUE_{FAA} = I_{FAA} / (I_{FAA} + M_{FAA}) \quad (5)$$

~~For results from analytical solution, Mooshammer et al. (2014) calculated ***NUE*** as:~~

~~$$NUE = (C_{FAA} - M) / C_{FAA} \quad (6)$$~~

~~Equation 6 implies that gross N mineralization derived from the analytical calculations is solely derived from FAA mineralization.~~

3. Results and discussion

3.1 Soil properties

Both investigated soils were acidic with pH of 3.7, but differed in other properties (Table 1). The Umbrisol had higher SOM and total C and N content, but lower C/N ratio. Nitrate concentration was below detection limit for both soils. The Umbrisol had prior to the ^{15}N addition a six-times higher FAA content compared to the Podzol and the relative abundance of individual FAAs differed as well between the two soils (Fig. 4). The FAA composition in the soil was initially dominated by acidic or non-aromatic compounds; possibly other FAAs might have been removed from the soil solution through plant root or microbe uptake (Andresen et al., 2011, Chen et al., 2015).

3.2 Analytical versus numerical approaches for quantification of gross N rates

Numerical tracing models represent robust methods to assess gross transformation rates, as all data points from the two isotope label experiments and all observed time steps are included. To our knowledge, quantification of total gross FAA mineralization and peptide depolymerization rates had not been done by numerical tracing models.~~To our knowledge, quantification of total gross FAA mineralization and peptide depolymerization rates had not been done by means of~~

~~numerical tracing models until now. However, these models represent robust methods to asses gross transformation rates, as all data points from the two isotope label experiments and all observed time steps are included.~~ A particular weakness of analytical approaches is that substrate addition stimulates consumption processes (Schimel, 1996; Di et al., 2000), which is also true for FAAs. This problem can be minimized by using numerical tracing models as the stimulation will be greatest immediately after ^{15}N labelling, but numerical models allow integration of transformation rates over a much longer period (Rütting et al., 2011). We indeed found that the numerical derived gross rates for M_{FAA} and I_{FAA} when integrated over 6 h, were several fold higher than rates integrated over the entire experimental duration (240 hours, Table 2; Fig. 5). Differences between gross rates derived from analytical and numerical models were greatest for FAA consumption, while smaller differences were found for D_{SON} and total mineralization (Table 2; Fig. 5). This points to an over stimulation of the processes by addition of FAAs, and demonstrate the advantage of longer incubation time with numerical data analysis to achieve more realistic gross rates.

From the Podzol we observed both FAA mineralization (M_{FAA}) as well as mineralization of other SON (M_{SON}). The total gross N mineralization rate (M_{FAA} + M_{SON}) derived from *Ntrace* integrated over 240 h was lower, but comparable to the analytically determined gross mineralization (M) rate (Table 2; Fig. 5). In this soil, FAAs mineralization contributed by only 12 to 15% to total gross N mineralization. For the Umbrisol, gross mineralization from *Ntrace* was only half the gross rate estimated by the analytical model and entirely assigned to M_{FAA}. The analytical model does not separate between mineralization from FAA or other N forms (M_{FAA} or M_{SON}), but provides one total rate (M). Quantification of FAA mineralization is possible using the analytical Eq. (4), ~~which though~~ but requires a ^{15}N tracing approach and two ^{15}N labellings (FAA and NH_4^+). ~~The analytical derived M_{FAA} (data not presented, Eq. 4) was in both soils higher than M (Eq.1 or 3), which is irrational. This might have been caused by the different time frames or the stimulation of M_{FAA} but not of M by the AA addition. In any case, numerical ^{15}N tracing models overcome such inconsistencies, as all gross rates are quantified simultaneously.~~

Depolymerization rates (D_{SON}) quantified by *Ntrace* were smaller compared to the analytical results for the Podzol, but these were similar for the Umbrisol (Table 2; Fig. 5). Gross depolymerization quantified by the analytical approach only had a minor decrease with increasing incubation time (Table 2; Fig. 5 c, d), suggesting no or only little re-mobilization of ^{15}N (Bjarnason, 1988). The similarity of D_{SON} rates quantified with *Ntrace* and by analytical approach confirms the validity of the numerical tracing model. The main difference between the two approaches is that the numerical approach estimates the rate for the entire 240 h of incubation, while the analytical approach considers a limited time span of max. 6 h.

3.3 Gross N dynamics in two contrasting forest soils

As the numerical *Ntrace* model is less prone to disturbance by ^{15}N label addition and as interactions between different N transformations are taken into account, we suggest that this approach provides more realistic gross N transformation rates. The ratio of total gross N mineralization (M) to peptide depolymerization (D_{SON}) rate ranges from 5 to 25 % in both organic

soils and plant litter, based on analytical calculations ~~rate ranges from 5 to 25 % in organic soils, based on analytical calculations~~ (Wanek et al., 2010; Wild et al., 2015). We found in both soils much higher ratio, being 76 % for Umbrisol and 170% for Podzol using the analytical approach, while *Ntrace* resulted in *M* to D_{SON} ratios of 46 % and 400 %, respectively. Thus, gross N mineralization was highly important for the N cycle and for making N available in the soil. Moreover, the M_{FAA} amounted to 46 % (Umbrisol) and 65 % (Podzol) of D_{SON} (Table 2; *Ntrace* Fig. 5). The finding of c. 50 % of depolymerized peptide N being further mineralized to NH_4^+ as well as the higher total mineralization than peptide depolymerization in the Podzol, suggest that peptide depolymerization is not the single major rate limiting step for the soil N cycle (Schimel and Bennett, 2004). Rather the results suggest that amino acid mineralization rate is a major part of the gross N mineralization as hypothesized, and can be considered as a co-limiting step for plant N availability in terrestrial ecosystems.

The Podzol was characterized by a lower peptide depolymerization rate compared to previously studied sub- and top-soils from forests and grasslands (Wild et al., 2015). The Umbrisol soil, being more N, SOM and FAA rich (Table 1), showed consistently higher gross N transformation rates (Table 2; Fig. 5). This agrees with the finding of a correlation of high N status and faster N cycling (organic and inorganic) across cold-temperate forests (Finzi and Berthrong, 2005). One pronounced difference between the two soils was the mineralization dynamics: for the Umbrisol the gross N mineralization was estimated as entirely derived from the FAA pool (100 % M_{FAA}), while in the Podzol M_{FAA} contributed only by 15 % to the total gross N mineralization (Fig. 5). Consequently, the Umbrisol strongly depended on FAAs as source for IN, while in the Podzol the mineralization of other organic N forms (M_{SON}) dominated the IN production. Notably, M_{FAA} was about ten-fold lower in Podzol compared to Umbrisol. This can be explained by the much smaller D_{SON} in the Podzol (Fig. 5), limiting the substrate for M_{FAA} in this soil, which is also reflected in the six-fold smaller FAA content (Table 1). Variation in the contribution of M_{FAA} to *M* has been reviewed previously, ranging from 35 % to 100 % across agricultural and natural soils, from results obtained using analytical calculations (Andresen et al., 2015). The C to N ratio for the two soils near 20, which indicates that the soils are at a tipping point for either C or N limitation, according to the concept from Mooshammer *at al.* (2014; Figure 1). Our result of amino acid nutrient use efficiency (***NUE***$_{FAA}$) was 0.57 for Umbrisol and 0.60 for Podzol, which point towards a carbon limitation in those soils, as we hypothesized.

~~**are connected to differences in soil organic matter quality and properties of the microbial biomass (Farrell et al., 2014). The C/N ratios for the two investigated soils were near the breakpoint (C/N ratio of 20) suggested by Mooshammer et al. (2014), at which a change from C limitation to N limitation of the microbial community occur (Fig. 6). By using the gross rates from *Ntrace*, the NUE_{FAA}s were 0.57 for Umbrisol and 0.60 for Podzol, which is smaller than expected from the relationship presented by Mooshammer et al. (2014) (Fig. 6). However, the *Ntrace* derived NUE_{FAA}s agree with the results from the analytical approach obtained from the longest time step (30 mins to 360 mins), but not for the shorter time steps (Table 2; Fig. 6). For Umbrisol the NUE_{FAA} from the analytical approach (Eq. 6) at the shorter time steps (30 min to 60 min and to 120 min) were higher and fell within the confidence interval from Mooshammer et al. (2014; Fig. 6). We account this to the fact that Eq. (6) uses gross FAA consumption rates quantified by the analytical approach. As it is well understood, this approach provides an overestimation of consumption rates (C_{FAA}), due to substrate addition (Schimel, 1996; Di et al., 2000), hereby, the *NUE* (Eq. 6) will be biased towards high values. The Podzol showed significant input to gross mineralization from other organic N than**~~

~~FAAs therefore, the *NUE* of Podzol derived from the analytical equation (Eq. 6) (time step 30 min to 120 min) was low. Consequently, NUE_{FAA} is ideally assessed by considering FAA mineralization explicitly (Eq. 5). If the true NUE_{FAA} is lower as we suggest from the *Ntrace* approach, it is likely that a larger portion of FAAs taken up by microbes is subsequently mineralized, than would be suggested from the line in Fig. 6. This challenges the understanding of the shift of soil C limitation to N limitation, however the two investigated soils can neither be termed as N or C limited.~~

3.4 Suggested improvements of the laboratory method

The FAA label addition was 10 to 20 times larger than the initial FAA content in the soil. Wanek et al. (2010) recommend adding maximum 25 % of the background amino acid content, but we were not able to reach the recommended level.~~Following the recommendation by Wanek et al. (2010), the FAA label addition was 10 to 20 times larger than the initial FAA content in the original substrate (litter or soil).~~ This specification requires pre-knowledge of the FAA content in the soils. The addition of FAAs might cause an unintended 'hot-spot' effect (Kuzyakov and Blagodatskaya 2015) which stimulates depolymerization~~,~~ by priming~~, and this is often difficult to avoid in such an experimental approach~~ (Schimel, 1996; Di et al., 2000). Furthermore, upon addition of high amount of amino acids, peptidases could be repressed (Vranova *et al.* 2013; Glenn *et al.* 1973). ~~Addition of a too small amount of FAAs would, however, potentially give enrichments of the individual FAAs at or below the detection limit and should be avoided.~~ Therefore, future studies s~~h~~could apply lower amounts of FAA~~, thereby further avoiding an unwanted stimulation of gross N rates~~.

Adsorption (physical-chemical) of the added label to SOM cannot be evaluated with our methodology, even when comparing: initial FAA, added FAAs and the FAA amount after 10 min time step (data not shown), because significant microbial N-transformations cannot be excluded (Jones et al., 2013). However, during the first time steps (30 min, 60 min and 120 min) only little change in ^{15}N % fraction was observed (Fig. 2 and 3), suggesting quite small depolymerization. ~~This point~~This point to the fact that there can be a limit to how small a peptide depolymerization rate we can measure with the current methodology. The individual FAAs were consumed equally through the time series as suggested by decreases in content (data not shown), and at the final time step, the individual FAA contents were back at the background level, hence, our procedure encompass a life-cycle for the added FAA quantity.

We did not assess peptide depolymerization rates for individual FAAs, because transformations between FAAs (Knowles et al., 2010) can neither be ruled out nor tested with our experimental set up (e.g. potential aspartic acid formation from asparagine or break down of larger FAAs such as lysine to smaller size such as serine). We aimed at quantifying gross rates relevant for organic N transformations in soils, using incubations with either ^{15}N-NH_4^+ or ^{15}N-AA mix label. Only the first sampling time point was synchronized for the two incubation types, we suggest that at least one more synchronized sampling ~~(e.g. after 6 h)~~ should be done in future experiments. Furthermore, after addition of ^{15}N labelled FAA, we observed a ^{15}N enrichment of NH_4^+ even at the last extraction (Fig. 2, 3). Therefore, future studies should include later extractions (e.g. at 48 h) to follow the fate of the added ^{15}N-AA. We expect that these suggestions would further improve the model estimation.

Finally, a further improved understanding of the FAA dynamics can be achieved by improving the analytical capacity for measuring all 20 proteinogenic FAAs. In this ~~paper~~ experiment we could not measure 4 of the added amino acids, but they were considered in *Ntrace* for realistic quantification of especially M_{FAA}. Assumptions had to be made for the ^{15}N enrichment of the non-measured FAAs and that the non-measured FAA concentration decreased like the measured FAAs. The quantified gross rates were not very sensitive to alteration of the ^{15}N enrichment of the non-measured FAAs (see Suppl. Table). We assumed similar ~~behavior~~behaviour as all 16 measured FAAs showed similar time courses in soil content after labelling (data not shown).

An even further development of the numerical model would include the NO_3^- dynamics in a soil with that property. Another outlook is that depolymerization rates of polymers other than amino acids (such as ~~chitin~~amino sugar polymers) are potentially an important part of the total depolymerization. Hence, further research is needed to uncover the importance of other limiting steps in the N-cycle.

Conclusively, we suggest that i) numerical modelling in conjunction with ^{15}N tracing ~~should be used when~~is an improvement for simultaneously determining FAA mineralization, peptide depolymerization and gross N mineralization rates ~~as a preferred alternative~~compared to the analytical equations which only determine one rate using data from only two time points; ii) FAA mineralization and FAA immobilization rates can be used for assessing FAA use efficiency (NUE_{FAA}) and soil N limitation~~FAA mineralization and FAA immobilization rates are used to determine microbial NUE_{FAA} as this gives a better estimation of NUE than if NUE is based on gross N mineralization (M) and C_{FAA}~~; iii) FAA mineralization might be as equally an important rate limiting step for gross N mineralization as peptide depolymerization rate is, because about half of all depolymerized peptide N is consecutively being mineralized and iv) depolymerization of other components in the soil is an additional potentially rate limiting step for the N cycle, which needs further investigation.

Author contribution

TR, LK, PB and LCA planned the experiments; AKB and LCA conducted the field work and lab-incubations, supervised by TR. SB conducted the stable isotope analysis. TR developed the *Ntrace* model. All authors discussed the conceptual model and contributed to data interpretation and the writing of the paper.

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Supplement Table S1.

Sensitivity analysis of different initial ^{15}N values for the 'missing' amino acid pool.

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Tables

Table 1. Soil properties for the two soil types: Podzol and Umbrisol from Skogaryd. Averages with standard error. pH (in 1 M KCl) Gravimetric soil water content (GWC), Soil organic matter (SOM), dry soil carbon (C) and nitrogen (N), C to N ratio of ~~dry~~ soil, soil content of NH_4 (µg g^{-1} DW) and total free amino acid content (FAA in µg N g^{-1}DW).

	Podzol	Umbrisol
pH	3.7 ± 0.0	3.7 ± 0.0
GWC (%)	34.2 ± 2.1	52.9 ± 3.8
SOM (%)	6.9 ± 0.4	9.3 ± 0.8
Dry soil C (%)	3.4 ± 0.2	4.7 ± 0.5
Dry soil N (%)	0.15 ±0.01	0.24 ± 0.03
C to N ratio	22.7 ± 0.4	19.4 ± 0.3
NH_4 (µg g^{-1} DW)	1.4 ± 0.6	1.1 ± 0.9
FAA (µg N g^{-1}DW)	~~1.3~~0 ± 0.~~6~~2	~~7.7~~0.4 ± 0~~3~~.1

Table 2. N dynamics rates from analytical equations (Eq. 1, 2, 3, 4 and 6) and *Ntrace* numerical model, average in [ng N g^{-1} h^{-1}] and standard deviation; NUE_{FAA} is dimensionless. Peptide depolymerization rate (D_{SON}); FAA immobilization rate (I_{FAA}); FAA mineralization rate (M_{FAA}); amino acid consumption (C_{FAA}); mineralization rate of organic N (M_{SON}); gross N mineralization (M); ammonium consumption (C_{NH4}); immobilization rate of NH_4^+ (I_{NH4}) and microbial amino acid nutrient use efficiency (NUE_{FAA}) (Eq. 5 ~~and 6~~). For D_{SON}, C_{FAA}, and NUE_{FAA} the time step 30 to 360 min is presented; M_{FAA} and I_{FAA} for all 20 AAs over 240 h. C_{FAA} and M from *Ntrace* are calculated sums ($M = M_{SON} + M_{FAA}$ and $C_{FAA} = I_{FAA} + M_{FAA}$).

	Podzol		**Umbrisol**	
	Analytical	***Ntrace***	**Analytical**	***Ntrace***
D_{SON}	58.8 (53.2)	18.2 (12.6)	316.6 (151.3)	288.5 (40.6)
I_{FAA}	-	16.8 (1.4)	-	172.3 (18.2)
M_{FAA}	-	11.2 (1.4)	-	131.7 (12.6)
C_{FAA}	313.8 (40.6)	28.0 (2.8)	851.6 (191.9)	303.9 (30.8)
M_{SON}	-	61.6 (5.6)	-	0.0
M	100.8 (35.0)	72.8 (7.0)	239.5 (135.9)	131.7 (12.6)
C_{NH4} (I_{NH4})	50.4 (11.2)	57.4 (5.6)	166.7 (198.9)	128.9 (19.6)
NUE_{FAA}	~~0.62 (0.39)~~	0.60 (0.12)	~~0.61 (0.86)~~	0.57 (0.12)

Figures

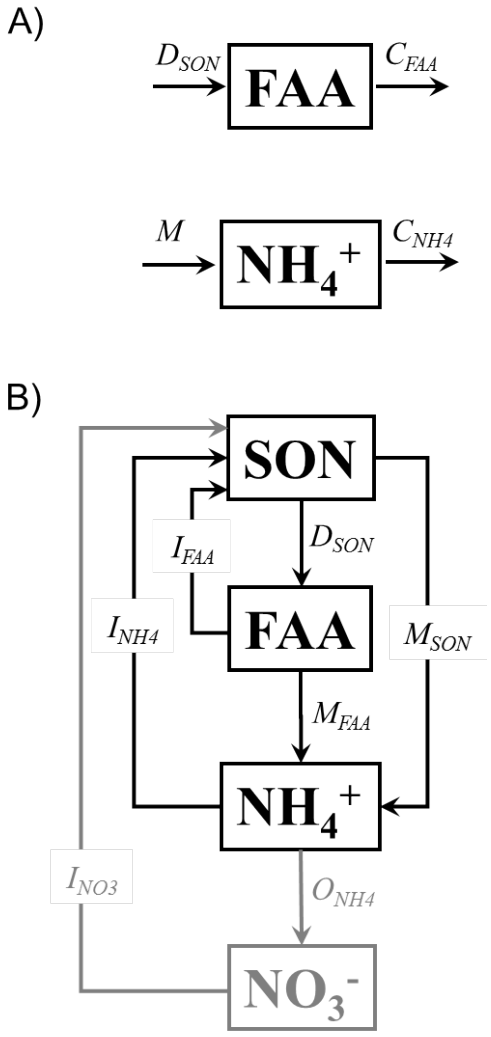

Figure 1. A) Schematic analytical model; Gross N mineralization (*M*); ammonium consumption (C_{NH4}); amino acid consumption (C_{FAA}) and peptide depolymerization rate (D_{SON}); B) The conceptual model *Ntrace* considers pools for: soil organic nitrogen (SON), free amino acid (FAA), ammonium (NH_4^+) and nitrate (NO_3^-), and fluxes of peptide depolymerization rate (D_{SON}), FAA mineralization rate (M_{FAA}), FAA immobilization rate (I_{FAA}), mineralization rate of organic N (M_{SON}), immobilization rate of NH_4^+ (I_{NH4}), NH_4^+ oxidation rate (O_{NH4}) and NO_3^- immobilization rate (I_{NO3}). Grey pools and fluxes could not be investigated in the current study due to too low nitrate content.

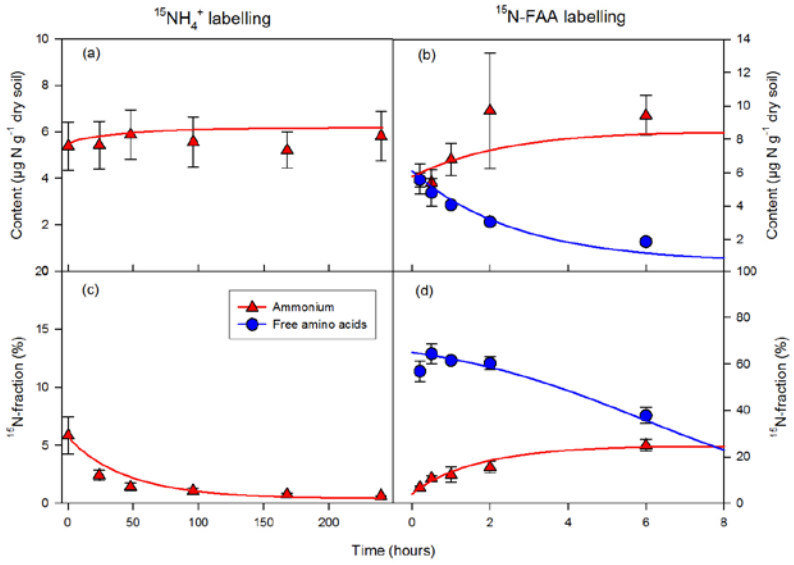

Figure 2. Umbrisol, time flow of the two labelling experiments: $^{15}N\text{-}NH_4^+$ labelling and ^{15}N-FAA labelling; symbols indicate data observation with standard ~~error~~deviation (n = 5; except ^{15}N fraction of free amino acids: n = 4 at 13 min), and lines indicate the two AA-pool model, where triangles and red is ammonium and circle and blue is FAAs. (a) and (b) N content [µg N g^{-1} DW soil] and (c) and (d) ^{15}N fraction (%).

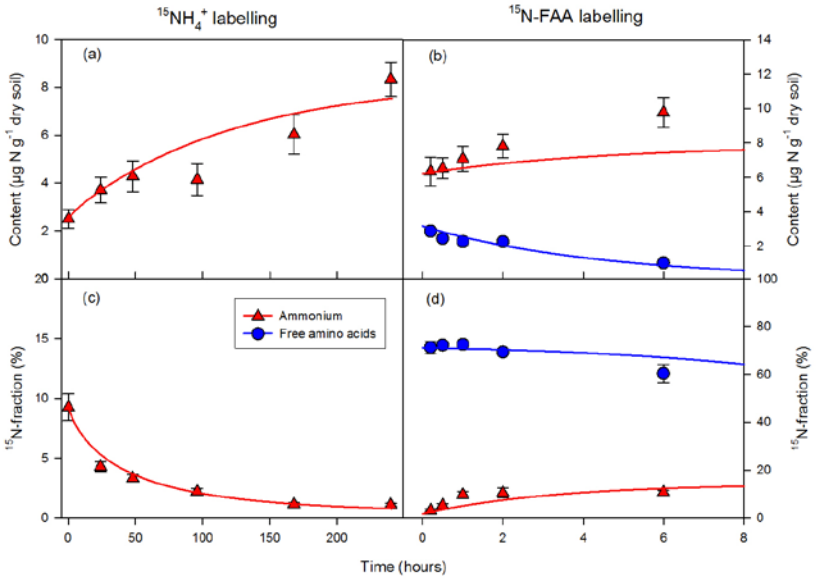

Figure 3. Podzol, time flow of the two labelling experiments: $^{15}N\text{-}NH_4^+$ labelling and ^{15}N-FAA labelling; symbols indicate data observation with standard ~~error~~deviation (n = 5; except ^{15}N fraction of free amino acids: n = 3 at 13 min), and lines indicate the two AA-pool model, where triangles and red is ammonium and circle and blue is FAAs. (a) and (b) N content [µg N g^{-1} DW soil] and (c) and (d) ^{15}N fraction (%).

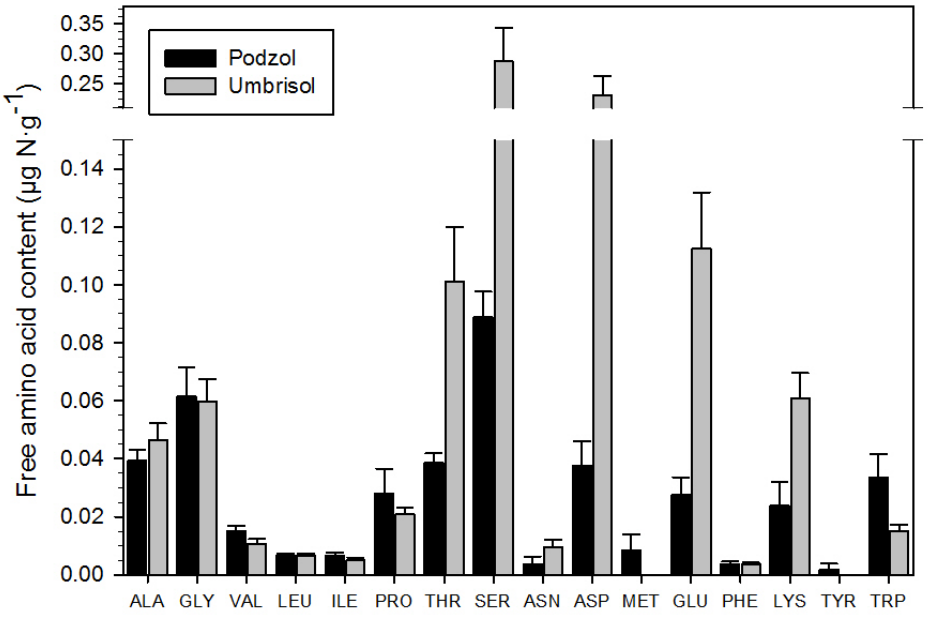

Figure 4. Initial soil content of individual amino acids (µg N-FAA g^{-1} DW soil) indicated as average ± standard error (n = 5).

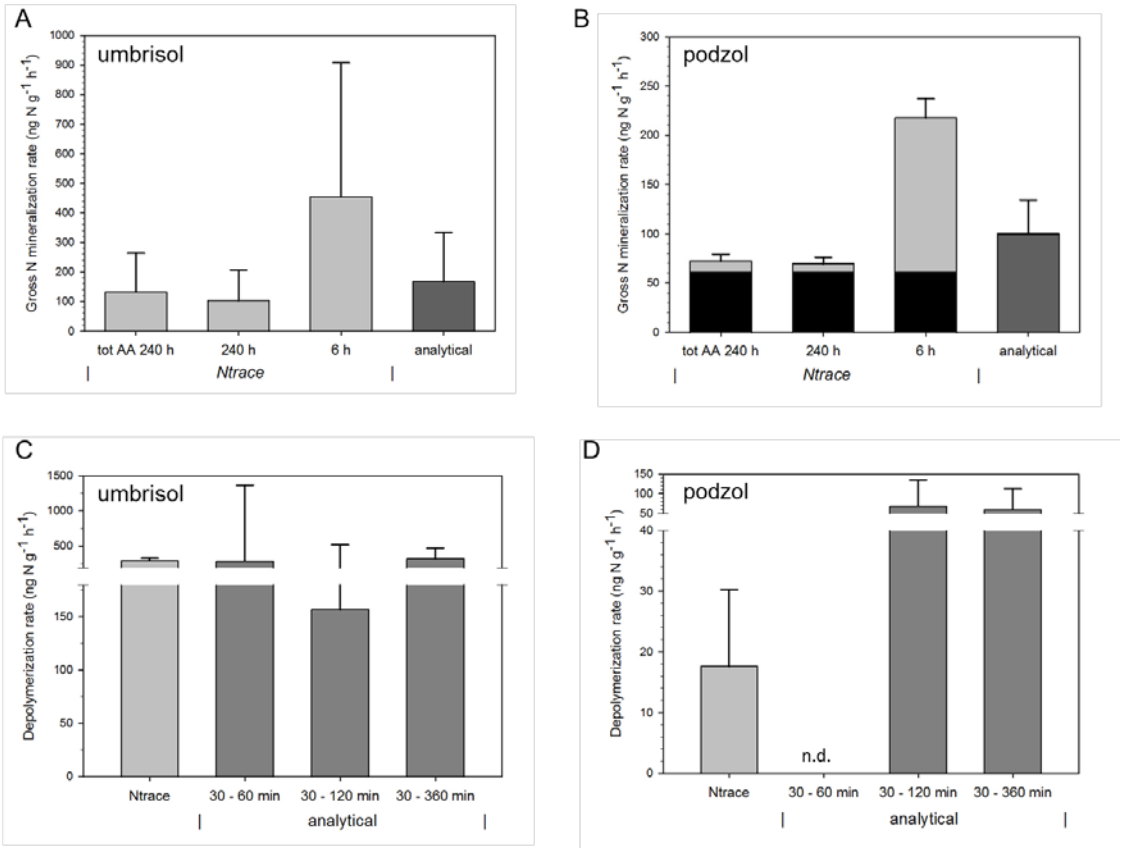

Figure 5. N transformation rates obtained by numerical modelling (*Ntrace*) and analytical equations. Gross N mineralization rates [n~~mol~~g N g^{-1} h^{-1}] indicated as average with deviation (n = 5) for Umbrisol (A) and Podzol (B); from the *Ntrace* model as sum of M_{SON} (black~~dark~~; note in Umbrisol M_{SON} is zero) and M_{FAA} (light grey): 'tot AA 240 h' is calculated for all 20 AAs over 240 h; '240 h' is calculated for the 16 measurable AAs over 240 h and '6 h' is calculated for the 16 measurable AAs for the initial 6 h; 'analytical': (dark grey) from the analytical equation for the time step 0 to 24 h. Depolymerization rate (total) in [~~nmol~~ ng N g^{-1} h^{-1}] as average with standard deviation (n = 5), for Umbrisol (C) and Podzol (D); from *Ntrace* model; or at the time steps: '30 - 60 min' (for Podzol this is not determined (n.d.) due to unchanged ^{15}N in all replicates), '30 - 120 min' and '30 - 360 min' from the analytical.

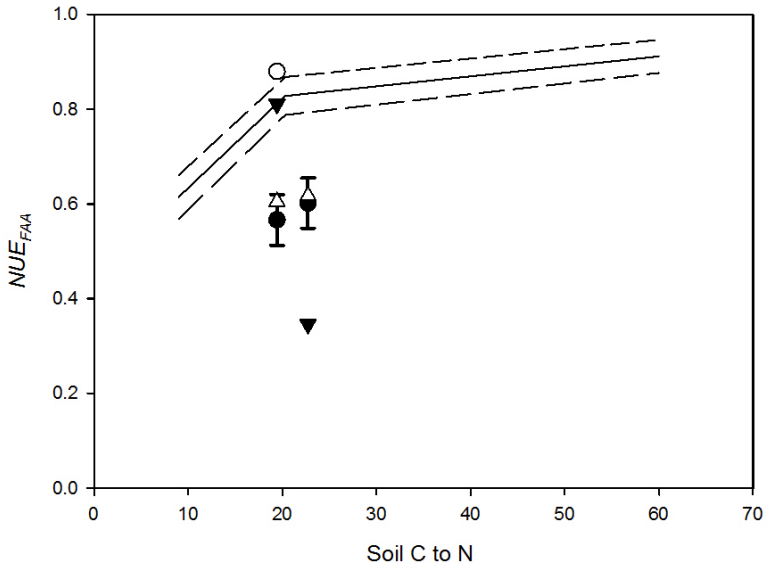

Figure 6. NUE_{FAA} **(microbial amino acid nutrient use efficiency) seen in relation to the soil C to N ratio (19.4** for Umbrisol and 22.7 for Podzol); NUE_{FAA} calculated by Eq. (5) upon numerical model calculation is filled circles; open circle is NUE from analytical calculated rates of AA consumption at time steps 30 to 60 min and gross N mineralization extrapolated to 6 h, using Eq. (6), for Podzol this was not determined due to unchanged ^{15}N in all replicates; filled triangles at time steps 30 to 120 min; and open triangle at time steps 30 to 360 min. The two pieced line of NUE vs. soil C to N ratio from Mooshammer *et al.* 2014 is regression line with standard error, from organic and mineral soils and plant litter calculated from Eq. (6).